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The impact of long-term pitching yeast storage on viability and fermentation performance

The purpose of this study was to evaluate which yeast storage conditions allow the pitching yeast to conserve maximum viability and fermentation performance. For that, a lager yeast cropped after a third fermentation cycle was subjected to different pretreatment and storage conditions during a three-month period. Pretreatment included washing the yeast with sterile process water. Storage conditions were varied by different gassing regimes with either air or carbon dioxide. Alternatives for an activation protocol prior to fermentation were also evaluated at laboratory scale after storage. Yeast was tested weekly for viability and microbiological purity. Additionally, a laboratory scale fermentation was performed monthly to evaluate the performance of the yeast from the different trials. Finally, the combination of the most promising pretreatment, storage conditions and re-activation technique was successfully tested on industrial scale. The availability of a large amount of pitching yeast complying with the required viability, fermentation performance and microbiological purity is a decisive advantage during the time-critical run-up procedure after a brewery shutdown. This may save the brewery several weeks before beer sales can resume.

Descriptors: pitching yeast, long-term storage, viability

1 Introduction

There is little information in literature about the best long-term storage conditions for cropped lager yeast in the brewery. It is generally accepted that temperatures between 0-5 °C, low alcohol and oxygen levels as well as agitation, may maintain the yeast viable and vital until the next pitching cycle, which should happen as soon as technically possible after cropping [4, 6, 7, 9, 11]. Ideally, no more than a 4-day storage time is recommended [3, 12].

In certain situations, the need to store yeast for longer periods may arise, thus a strategy to maintain the yeast viable in order to perform an efficient restart of the plant activities is required. Low temperatures, while avoiding freezing conditions, are a well-known measure to keep metabolism low [5, 8, 10]. Additionally, the removal of stress factors has been shown to have a positive effect on yeast viability and vitality. It is common practice to use periodic aeration to achieve this goal [2, 12, 13].

Ethanol and carbon dioxide are considered to be the most relevant stress factors during yeast storage [1, 2, 4]. Aeration serves to homogenize the yeast in its storage vessel and additionally, this mixing process helps to maintain the yeast cold, especially in the center of the vessel, far away from the cooling jackets [3, 12].

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The purpose of this study was to evaluate which yeast storage conditions allow the pitching yeast to conserve maximum viability and fermentation performance over the course of several months. For this purpose, both analytical methods as well as yeast handling procedures have been used which are simple enough to be reproduced in most breweries.

2 Materials and methods

First, lab scale assays where performed using washed and non-washed standard Polar brewery lager yeast (*Saccharomyces pastorianus* ssp. *carlsbergensis*, a type 34/70 derivative) to assess the effect of ethanol in the long-term viability of cropped yeast. All assays where done in duplicates.

Table 1 Aeration regimes used in the lab-scale assay with both washed and non-washed yeast

Treatment	Aeration	Interval
1	Air	Every 24 hours
2	Air	Every 72 hours
3	CO_2	Every 72 hours
Control	None	–

Table 2 Wort concentration and aeration used in the activation assay

Treatment	Aeration time (min)	Wort concentration (% w/w)
1	30	–
2	30	0,5
3	30	5
Control	–	–

The washing procedure was applied to 100 g of 3rd cycle lager yeast, using sterile process water in a 1 : 4 proportion by weight. After thorough blending with a magnetic stirrer, this mixture was left to sit for 24 hours at 3 °C, the supernatant was removed by decantation and the washed yeast, left in the bottom of the beaker, was used in the subsequent assays. Alcohol was measured in the yeast before and after the washing procedure.

Different aeration regimes were established, using both air at different intervals, as well as carbon dioxide gassing at 0,1 – 0,2 bar for the purpose of yeast homogenization and cooling using 1 L Büchner flask. The different aeration regimes used are described in table 1.

Yeast slurries were maintained for 3 months between 1 – 3 °C. A non-homogenized control was added for academic purposes, since aeration is needed to maintain the temperature homogenous in our large-scale yeast storage vessels. The results of this non-aerated trial may be relevant for breweries whose yeast vessels are equipped with stirrers, however. The 24-hour aeration regime was selected since this is the standard treatment at the brewery in normal operative conditions. Brewery measurements have shown that before 72 h, no significant temperature increase was detected in the center of the yeast storage vessel. Therefore, air and CO_2 gassing was also included every 72 h.

Different analyses were carried out to assess the physiological state of the yeast, including weekly analysis of the viability using the cell count method, monthly lab-scale fermentations and weekly microbiological controls for lactic acid bacteria and wild yeasts.

The cell count method was done with a 1/100 serial dilution of the stored yeast, which was mixed, in a 1 : 2 proportion, with a 10 % solution of methylene blue (Merck KGaA, Darmstadt, Germany), the count was made in a Neubauer chamber. Microbiological controls were carried out by the spread plate method in NBB agar (Döhler GmbH, Darmstadt, Germany) for lactic acid bacteria and yeast and mold agar (Oxoid, Hampshire, UK) + 195 ppm CuSO4 (Merck KGaA, Darmstadt, Germany) for wild yeasts.

Lab scale fermentations were performed in a 1 L sterile cylinder with 500 mL of 14 % w/w extract wort, produced at industrial scale in the brewery. In a 20 °C environment, a proportion of 1 x 10^6 cells per % extract was used to calculate the amount of yeast needed to pitch. Apparent extract (% w/w), alcohol (% v/v) and pH were measured daily until apparent extract was stable, indicating that fermentable sugars where completely consumed.

Alcohol and extract were determined with an Alcolyzer Beer ME and Beer Analyzer DMA 4500M, respectively (Anton Paar GmbH, Graz, Austria) and pH was measured, after degassing, using an Orion 3 Star pH meter (Thermo Fisher Scientific Inc., Beverly, USA).

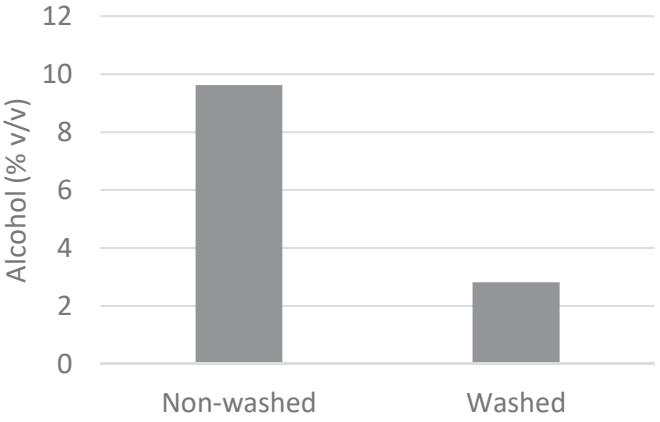

Fig. 1 Alcohol values before and after the washing procedure of cropped yeast

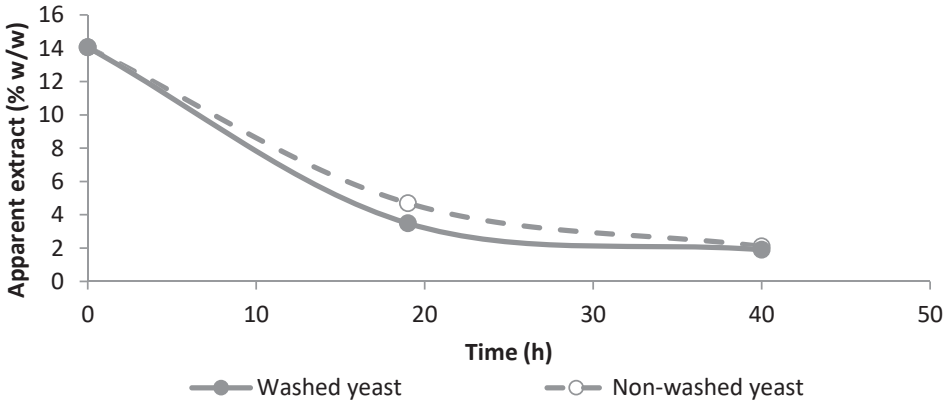

Fig. 2 Extract attenuation in initial lab scale fermentation for washed and non-washed yeast

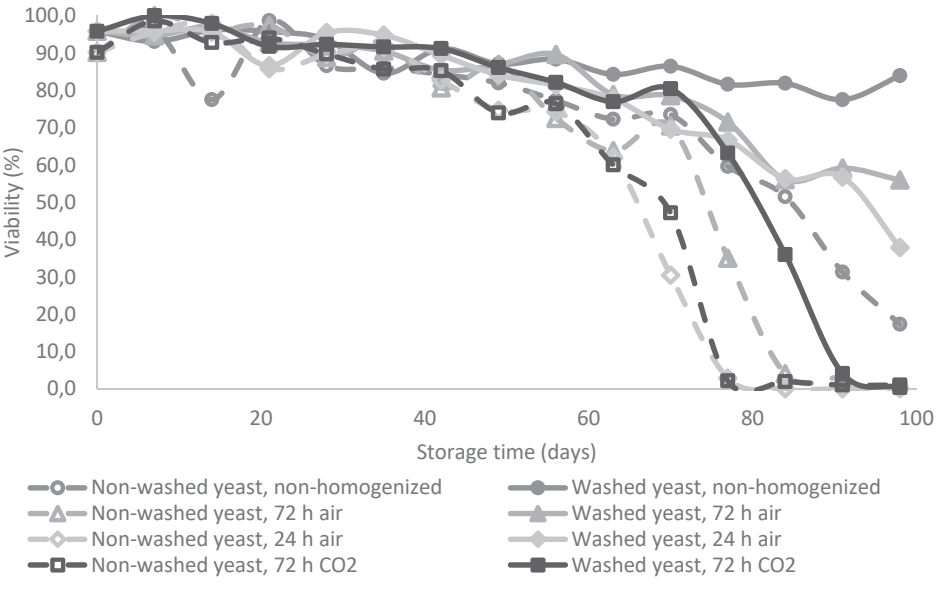

Fig. 3 Yeast viability values for each aeration treatment evaluated

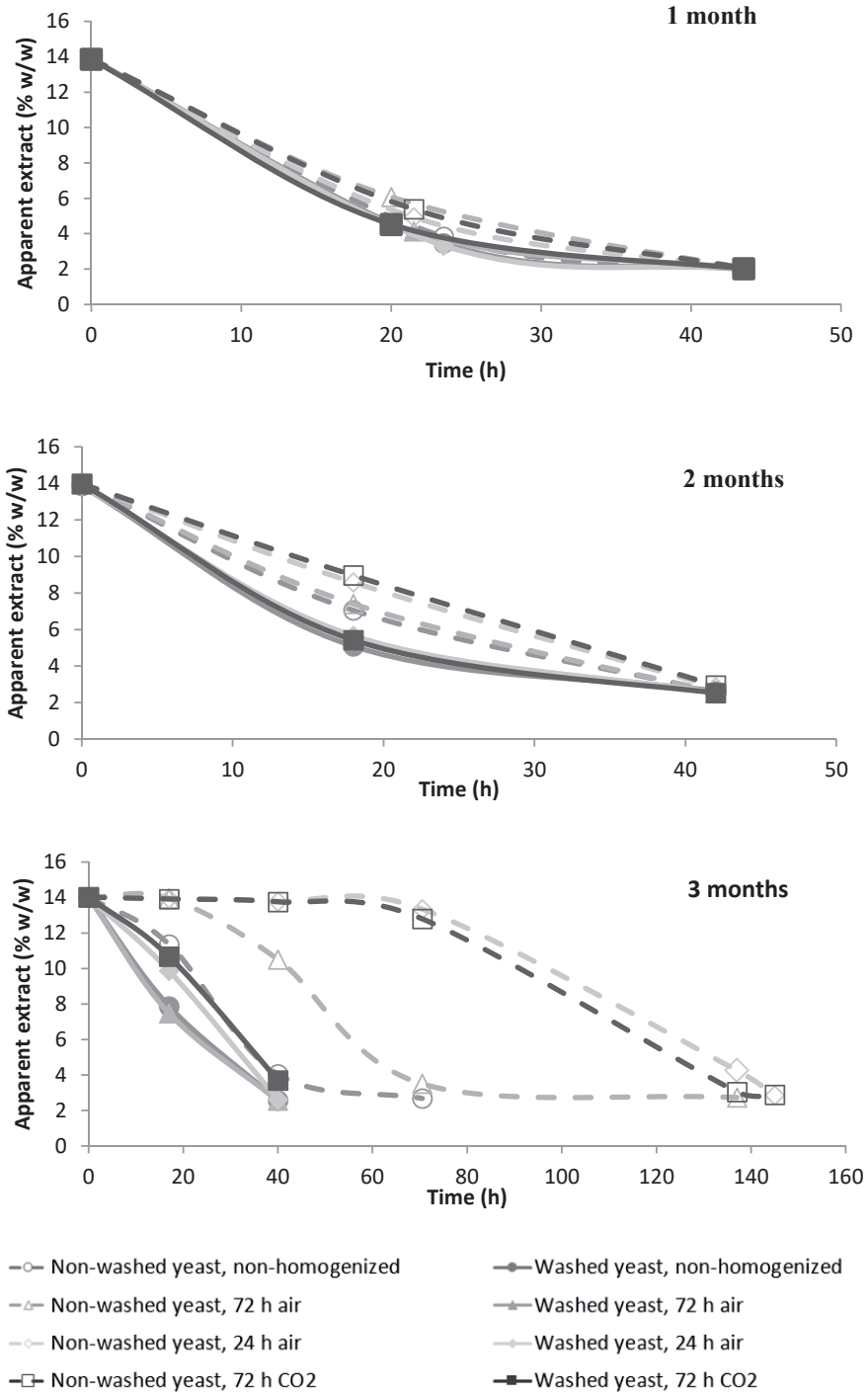

Fig. 4 Extract attenuation in lab scale fermentation for each treatment evaluated

Besides the selection of suitable yeast storage conditions, the design of an effective yeast reactivation step was a goal. The purpose of this treatment is to give the stored yeast the capacity to start the fermentation process promptly. For this, a washed yeast specimen stored for a 3-months period was used. The yeast was subjected to aeration during 30 minutes and subsequently exposed to different wort concentrations. The conditions evaluated are shown in table 2. The resulting yeast performance was evaluated through a lab scale fermentation, as described above.

After both storage and reactivation methods had been selected with the help of lab-scale assays, an industrial-scale assay was done to verify its applicability. A lager yeast (150 hl) was washed in a 6500 hl cylindroconical tank (CCT) with sterile water (1 : 4) and left to sediment for 24 hours at 3 °C. It was cropped afterwards, using standardized operational procedures, and was transferred to a 200 hl yeast vessel where it was aerated every 72 hours with sterile air, at 0,1 – 0,2 bar, for a 3-month period. Microbiological controls where done weekly.

After the 3-month period, an activation step was done adding 20 hl of 5 % wort, followed by a 4-hour aeration. Yeast was left for 4 hours to sediment and was then employed to pitch a CCT. An industrial fermentation was done with 14 % extract wort. Apparent extract, alcohol, and microbiological controls were measured as described above.

3 Results and discussion

Lab-scale assays were carried out to determine the best parameters to maintain cropped yeast for long periods of time. First, a cropped lager yeast was washed to remove alcohol, the results before and after this procedure are shown in figure 1. In addition, an initial fermentation was done with these two yeasts for further comparison, the results are shown in figure 2. Even with the significant differences seen in the alcohol levels, 9,6 % before washing vs 2,8 % after washing, the fermentation performance was similar between the two yeasts.

During the storage period, viability of the yeast was measured for each treatment. Results are presented in figure 3. A consistent decrease was observed for all the non-washed yeast trials, independently of the aeration regime used, after 60-days of storage. For the washed yeast, the decline in the viability values below 70 % started after the 80th day for all the treatments except the control without homogenization, which showed an 84 % viability after storage for 98 days. The second regime with the lower impact in yeast viability, was aeration with air every 72 hours resulting in a 56 % viability after 98 days. This decrease in viability is expected as breakdown of glycogen occurs and ethanol is produced, therefore affecting the integrity of the cell. Also, oxidative stress may be happening and trehalose consumption may be limited by the use of low temperatures for storage [1, 10]. No microbiological contamination was detected in all the storage period.

In order to assess the vitality of the stored yeast, lab scale fermentations were performed every 30 days and their results are shown in figure 4. Each month, a slower rate of attenuation was observed for the non-washed yeast. After three months, complete fermentation was achieved after 140 hours, except for the control with no homogenization. Washed yeast completed the fermentation in no more than 40 hours. No difference was observed between aeration with air every 24 or 72 hours and the non-homogenized washed yeast. A slightly smaller rate of fermentation was observed in the CO_2-gassed trial.

In addition, alcohol levels were measured after the 90 days of storage for each treatment. An increase was observed in all cases in comparison with the initial levels for both the washed and non-washed yeast (Fig. 5). Higher levels were obtained for the non-washed yeast and the washed and CO_2-homogenized yeast. This increase, in comparison to the initial value, ranged between 15 % for the non-homogenized yeast and 80 % for the 24 hour aerated yeast. Among the washed yeasts, the highest alcohol increase was with the CO_2-gassed yeast with 16,4 % alcohol and the lowest was with the non-homogenized yeast with 6,0 % which doubled the initial value.

The increase in alcohol content observed was somewhat surprising in the trials where repeated gassing, with either air or CO_2, was applied. It is generally believed that aeration does not only remove CO_2, but also alcohol from the stored yeast. However, figure 5 shows that no removal of alcohol from yeast by means of gassing could be achieved, as all alcohol values after storage and gassing are higher than the initial alcohol content. One could argue that aeration promotes yeast metabolism and helps increase the alcohol level as a consequence, but then the CO_2-gassed yeast should have had a lower alcohol content, which is not the case.

The decrease in fermentation rates and also the increase in alcohol levels, suggest that the glycogen reserves are being depleted by the cells and therefore viability and vitality of the yeast are compromised [2, 10, 11].

Since non-washed yeast showed a poorer performance, it is probable that ethanol stress is impacting more negatively than any other factor, as concentration in all cases was above 7 % [1, 4, 12]. Some authors have suggested that dissolution in sterile cold water or wort, may improve the viability of yeast cells during storage for long periods of time, nonetheless water quality may be an obstacle to maintain microbiological purity of yeast [1]. In this study, removing the water after a washing procedure, proved to be a good alternative to dissolution.

It also should be noted that a strain similar to the standard Polar lager yeast used in this study has shown better ethanol resistance than several common ale strains [2] and that the overall performance may also be strain-dependent [12].

According to the results, a non-homogenization treatment provided better viability, less alcohol production and a good fermentation performance, which should lead to a better quality beer in terms of turbidity, flavour production and foam stability [4, 11]. Nonetheless, under industrial conditions, keeping the temperature homogeneous cannot be done without aeration, and some authors have suggested that the impact of so-called “hot spots” can be more detrimental to the stored yeast than the presence

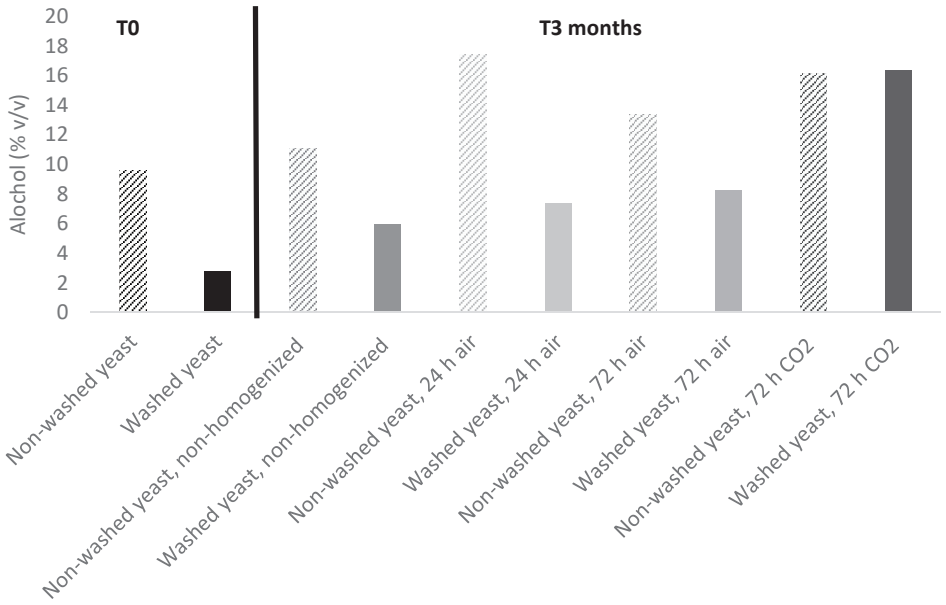

Fig. 5 Alcohol values for cropped yeast subjected to different aeration regimes after 90-days storage (T3 months). Initial values (T0) for both washed and non-washed yeast are presented for comparison

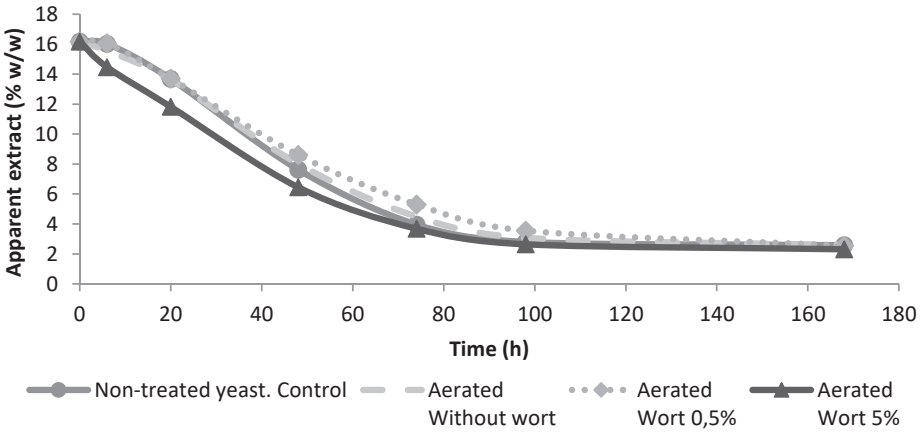

Fig. 6 Extract attenuation in lab scale fermentations for each activation treatment evaluated

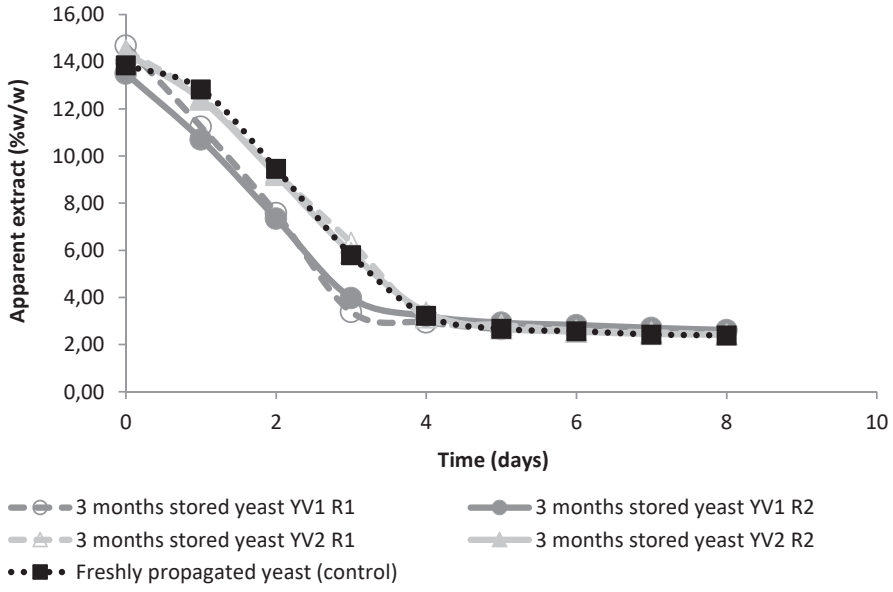

Fig. 7 Extract attenuation in industrial scale fermentation in a 3-month stored yeast aerated every 72 hours. Fermentation of a freshly propagated yeast is presented for comparison (YV: yeast vessel R: repetition)

of oxygen, since intracellular glycogen and trehalose decrease as temperature increases [9].

Even though these results suggest that the washed and non-homogenized treatment maintained the yeast in a better condition for a 90 days' period, nonetheless a 72-hour aeration with air regime was chosen for temperature control on industrial scale.

After the treatment had been selected, an activation protocol was needed in order to initiate the fermentation as fast as possible. A 3-month stored yeast was exposed to different wort concentrations and aerated for 30 min, a non-aerated yeast was used as control. The results are presented in figure 6, where all the treatments showed similar attenuation rates. The aerated yeast with an addition of 5% wort to an extract level of (1,58 ± 0,10) % (w/w) showed the fastest attenuation rate. Consequently, a 5 % wort concentration was selected for the industrial activation protocol. Higher extract values have not been considered in order to avoid an osmotic shock [14]. To ensure a complete homogenization of the yeast vessel and removal of CO_2, the aeration time was incremented to four hours according to our own experience on the industrial level and as well confirmed by *Wackerbauer* et al. [15].

Finally, industrial scale fermentations were carried out with 90-day stored yeasts from two different yeast storage vessels that had been aerated and activated with the selected protocols, in order to evaluate the performance under regular brewery conditions. The results show that good fermentation performance could be observed in all the CCTs evaluated, even compared with a freshly propagated yeast used as control (Fig. 7).

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4 Conclusion/Summary

In summary, yeast washing prior to storage has been found to be highly advantageous and our results have shown that its effect is far more important than aeration. The best treatments to keep yeast viable and vital for at least 3 months at laboratory scale were homogenizing with sterile air every 72 hours or not homogenizing at all. A pre-activation protocol was selected and the one that showed the fastest rate of fermentation was aerating the yeast plus an addition of 5 °P wort to an extract level of (1,6 ± 0,10) % (w/w). The procedure was tested at industrial scale, selecting aeration regimes that allowed temperature control and a homogenized yeast. After storing washed lager yeast at 1 – 4 °C for 3 months with aeration every 72 hours, followed by pre-activation with aeration and wort addition, satisfactory results were obtained. Fermentation performance of stored yeast was comparable to freshly propagated yeast.

Temporary brewery closures may occur in different parts of the world. They can be caused by many reasons including (but not limited to) economic downturns, availability of raw materials and energy, logistical problems, social unrest, war, natural disasters and pandemics. Once these factors have been removed, the availability of a large amount of pitching yeast complying with the required viability, fermentation performance and microbiological purity is a decisive advantage during the time-critical run-up procedure after a brewery shutdown. This may save the brewery up to four weeks of lead-time before resumption of beer sales.

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